p.(L576P) -*KIT* mutation in GIST: favourable prognosis and sensitive to imatinib?

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Abstract

Exon 11 *KIT* mutations are found in a majority of gastrointestinal stromal tumours (GIST) and are usually predictive of response to imatinib, a KIT, PDGFRA and ABL inhibitor. Exon 11 mutations with poor sensitivity to imatinib and poor outcome can be observed on rare occasions, including p.(L576P). *In silico* and *in vitro* studies suggested a decreased binding affinity for imatinib in p.(L576P) *KIT* mutations, thereby offering an explanation for their poor outcome and poor response to standard therapy. These observations were further corroborated with anecdotal case reports of refractoriness or non-durable response to imatinib therapy. However, we describe the favourable response to imatinib and outcome in five p.(L576P)-*KIT* mutant GIST patients treated at a tertiary sarcoma referral center. The sensitivity of p.(L576P)-*KIT* mutations to imatinib, and the prognostic impact of this mutation need to be further evaluated in a larger cohort. Based on our observations, p.(L576P) mutated GISTs should be treated with standard first line imatinib therapy.

Keywords: GIST, imatinib, mutation, L576P, dasatinib

Abbreviations list

GIST: Gastrointestinal stromal tumors

HPF: High power field

CE-SSCA: Capillary electrophoresis single-strand conformation analysis

RECIST: Response Evaluation Criteria In Solid Tumors

Introduction

A majority of gastrointestinal stromal tumours (GISTs) harbour *KIT* activating mutations, leading to constitutive ligand-independent activation of the tyrosine kinase receptor and subsequent tumor growth.[1] The introduction of imatinib, a PDGFRA, ABL and KIT inhibitor, has revolutionized systemic therapy and dramatically impacted survival.[2-4] As molecular profiling of GISTs is routinely incorporated into practice, an increasing number of mutations are being discovered. The most common mutations are found in exon 11 and affect the juxtamembrane domain of KIT, which prevents the activation loops from adopting an active conformation.[5] The end result is receptor dimerization in the absence of a ligand. Described mutations include single or combination substitutions, in-frame deletions and insertions. Exon 11 mutations are usually predictive of imatinib sensitivity with some exceptions. Among these is the rare missense mutation c.1727T>C leading to p.(L576P). An initial report suggested that this *KIT* mutation is associated with an aggressive clinical course and poor sensitivity to imatinib, which was further corroborated with *in silico* studies.[6] Recently, a large retrospective study on the clinical significance of *KIT* mutation, demonstrated a favourable relapse-free survival in patients with the p.(L576P) mutation.[7] Our experience at the Royal

Marsden also further supports the favourable prognosis of this subset of patients in addition to observing sensitivity to imatinib. We hereby describe the favourable outcome and response to imatinib therapy in five patients diagnosed with a p.(L576P) *KIT* mutated GIST.

Patients and methods

The histological diagnosis of GIST was confirmed by a soft tissue pathologist and by immunohistochemical staining of CD117 and DOG1 (K.T). For resected GISTs, the largest tumour diameter and mitotic count per 50 high-power fields (HPF) were evaluated after surgery to establish a risk category according to the Miettinen and Lasota risk stratification model.[8] Molecular analysis for *KIT* and *PDGFRA* mutations were carried on formalin-fixed paraffin embedded samples using capillary electrophoresis single-strand conformation analysis (CE-SSCA) followed by confirmation using bi-directional Sanger sequencing. Prior to commencing this study approval was obtained from the institutional review board and local ethics committee. A retrospective search of a prospective database was performed to identify GIST patients with *KIT* p.(L576P) mutations.

Case 1.

A 74 year old woman initially presented in 2009 with anemia and weight loss and was subsequently diagnosed with a localised gastric GIST. The patient proceeded to a partial gastrectomy in March 2009. Pathology review identified an 8.5 cm gastric GIST with a mitotic count of 20 per 50 HPF, corresponding to a high-risk category according to the Miettinen classification. Molecular

analysis confirmed the p.(L576P) *KIT* mutation. The patient was offered adjuvant imatinib (400 mg once a day) therapy for a 3 year period but declined. The patient was put on active surveillance and remains with no evidence of disease recurrence 6 years since her initial diagnosis.

Case 2.

A 54 year-old man, diagnosed with chronic lymphocytic leukemia was found to have a 7 cm mass outside the posterior wall of the bladder on routine surveillance. An initial biopsy in 2009 complicated by haemorrhage led to an emergency laparotomy with morcellation of the mass. Histopathology examination documented the presence of a > 5 cm GIST with a mitotic count of > 5/50 HPF, classified as high risk. Mutational analysis confirmed the p.(L576P) *KIT* mutation. The patient was commenced on imatinib 400 mg once a day with a subsequent good radiological response and near complete remission. In 2011, resection of residual disease was attempted, but failed due to multiple adherences. No residual tumor was found on histological examination. The patient continues on imatinib and remains well with no measurable disease on his latest scan (March 2015), six years since his initial diagnosis.

Case 3.

A 46 year old man was diagnosed in November 2012 with a locally advanced rectal GIST involving the prostate. Biopsy and subsequent mutational analysis confirmed the presence of a GIST with infrequent mitoses and the p.(L576P) *KIT* mutation. Pelvic exenteration was deemed necessary to resect the primary tumor. The patient was therefore put on neo-adjuvant imatinib 400 mg once a day. Fourth months later, the tumor had a marginal decrease in size

but remained stable by Response Evaluation Criteria In Solid Tumors (RECIST) criteria. In June 2013, imatinib was subsequently increased to 400 mg twice a day. Two months following the increase, the disease remained stable. Based on *in vitro* data concerning the p.(L576P) mutation, the patient was switched to dasatinib 50 mg twice a day. Repeat imaging continued to show stable disease, although there was a marginal decrease in the component of the tumour involving the prostate. On last-follow up, the patients remains on dasatinib and maintains stable disease, 3 years following his initial diagnosis.

Case 4.

A 28 year-old man presented with abdominal distension and gastrointestinal bleeding in October 2013. Imaging showed a large duodenal mass measuring 9 cm with multiple liver deposits. Core biopsies and molecular analysis confirmed a p.(L576P) *KIT* GIST with a low mitotic count (< 5 per HPF). The patient was put on imatinib 400 mg once a day and obtained a partial response by RECIST on subsequent imaging. A response plateau was reached in May 2014. Because of the possibility of obstruction if there were progression of the primary tumor, the patient underwent a Whipple's procedure in June 2015. On pathological examination, 20% of the tumour remained viable. The patient remains well with RECIST stable disease on imatinib therapy, 2 years since his initial diagnosis.

Case 5.

A 59 year-old woman experienced bloating and fatigue and was found to have a localised 14 cm gastric GIST in 2009. On biopsy, no definite mitoses were identifiable. Initial mutational analysis was not done. The patient was started

on neo-adjuvant imatinib 400 mg once a day, but had poor compliance because of significant toxicity. Five months after the start of therapy, the disease was stable by RECIST and subsequently underwent distal subtotal gastrectomy, right extended hemicolectomy and omentectomy. The post-operative specimen documented an abundant amount of necrosis (>50%) and a mitotic index of 8/50 per HPF, thereby classifying the disease as high risk. Mutational analysis confirmed the presence of the p.L576 *KIT* mutation. Because imatinib was initially poorly tolerated, she was not offered adjuvant therapy but instead was put on surveillance. The patient remains free of disease, now 6 years following her initial diagnosis.

Discussion

Our experience has demonstrated that p.(L576P) *KIT* mutated GISTs can respond to imatinib therapy and in addition are associated with favourable outcome, further supporting the findings by Joensuu et al.[9] These observations contrast with the poor response to imatinib and negative outcome in 2 patients described by Conca et al.[6] and 1 patient described by Antonescu et al.[10] *In silico* studies suggest that the p.(L576P) mutation induces conformational change similar to the active form of native KIT thereby explaining the decreased binding affinity of imatinib. *In vitro* drug testing on *KIT* p.(L576P) mutated anal melanoma cell lines also demonstrated that a 10-fold higher dose of imatinib is required to observe drug sensitivity compared to *KIT* imatinib sensitive mutations in addition to showing that dasatinib was particularly active.[11] However, *in vivo* responses to imatinib therapy were observed in melanoma patients harbouring the L576P mutation thereby demonstrating that *in vitro* testing does not necessarily correlate with *in vivo*

experience.[12] Furthermore, it is difficult to draw direct conclusions on drug sensitivity in p.(L576P) *KIT* mutated GISTs based on p.(L576P) *KIT* mutated anal melanoma cell lines. In addition, in our experience, no significant increase in radiological response was observable after switching from imatinib to dasatinib therapy in Case 3 despite in vitro data indicating increased drug activity. Finally, the adverse outcome and poor response to imatinib previously reported in anecdotal cases of p.(L567P) *KIT* mutated GIST may be related to alternative activating signalling pathways, mutations and host factors responsible for primary resistance. Commonly, early resistance to imatinib is observed in KIT exon 9, PDGFR, and wild-type GIST.[13] However, in recent years, additional mechanisms responsible for primary resistance in KIT exon 11 GIST have come to light. Variable interindividual drug metabolism may contribute to lower imatinib plasma levels and subsequent clinical responses in a subset of patients with KIT exon 11 GIST.[14] Furthermore, concomitant activating mutations in *KRAS* and *BRAF* genes were also involved in documented cases of primary resistance in KIT sensitive mutated GIST.[15] Finally, overexpression of CCND2 and EGFR, impaired p53, p16, BCL2, CHK2 expression, and silencing of CDKN2A, CDKN2C, SMARCB1, PTEN and DMD may all variably contribute to acquired resistance to imatinib therapy.[16-18] Further work is needed to elucidate the role of these alternative signalling pathways.

Based on our observations, standard dose imatinib may be sufficient to induce a response in p.(L576P) *KIT* mutated GIST, however, the number of patients is small and needs to be correlated with a larger series. *In vitro* drug

sensitivity screens on p.(L576P) *KIT* induced mutated GIST cell line would be helpful to better understand KIT tyrosine kinase inhibitor activity.

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